A xenon-arc lamp-based charge-coupled device (CCD) camera system for multispectral imaging in proteomics.

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1 Introduction

The convergence of advanced imaging instrumentation and small molecule detection reagents has created new capabilities in electrophoretic detection, especially for nonradioactive protein expression and post-translational modification (PTM) analysis. Fluorescent total protein stains and PTM stains provide detection limits matching silver staining but offer better dynamic range of quantification and improved compatibility with mass spectrometry. We describe an improved, newly introduced CCD camera-based imaging instrument, equipped with both a high pressure Xenon arc lamp and ultraviolet (UV) transilluminator, which provides broad-band wavelength coverage (380-700 nm and UV). With 6-position filter wheels, both the excitation and emission wavelengths may be selected, providing optimal measurement and quantitation of virtually any available dye and allowing excellent spectral resolution between different fluorophores. By acquiring images in succession, as many as four different fluorescent labels may be viewed from any single gel. The platform is shown to be suitable for imaging the wide range of colored and fluorescent dyes commonly encountered in proteomics investigations. Unlike conventional fixed CCD camera systems, the flexible new ProXPRESS 2D Imager is capable of scanning large areas at high resolution and providing accurate selectable illumination over the entire UV/visible spectral range, thus maximizing the efficiency of dye multiplexing protocols, a central strategy in proteomic analysis.

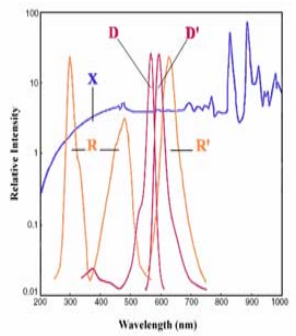
2 Instrument settings

All fluorescent stains were imaged using the top illumination mode of the ProXPRESS 2D Proteomic Imaging System (PerkinElmer). SYPRO Ruby stain was imaged using 460/80 nm excitation and 650/150 emission filters. SYPRO Orange stain was imaged using 460/80 nm excitation and 590/30 nm emission filters. Nile Red dye was imaged using 540/25 nm excitation and 590/30 nm emission filters. Pro-Q Diamond stain was imaged using 540/25 nm excitation and 590/30 nm emission filters. A white light UV-to-visible converter was employed for imaging of Coomassie Blue and silver stained gels. Both stains were imaged by bottom illumination, using the neutral density filter. Images were typically acquired at 100 µm resolution setting.

ProXPRESS 2D Imager

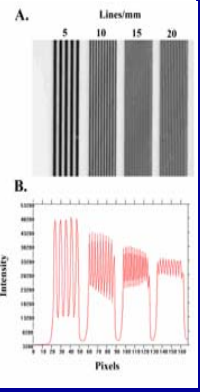
3 Compatibility with small and large Stoke's shift fluorophores.

The figure schematically depicts the spectral output of the Xenon arc lamp employed in the ProXPRESS 2D imager relative to the excitation/emission profiles of two commonly employed dyes in proteomics, SYPRO Ruby protein gel stain and Pro-Q Diamond phosphoprotein gel stain (Molecular Probes). X = Spectral output of the 300 watt Xenon-arc lamp. R = Excitation peaks of SYPRO Ruby stain. R' = Emission peak of SYPRO Ruby stain. D = Excitation peak of Pro-Q Diamond stain. D' = Emission peak of Pro-Q Diamond stain. SYPRO Ruby stain is a large, while Pro-Q Diamond stain is a small Stoke's shift dye. *Through judicious choice of filters, virtually any fluorescent dye can be optimally imaged on the instrument.*



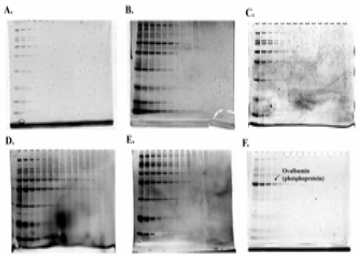
4 Resolution of imaging system

The ProXPRESS 2D imager employs a high performance 3.2 million pixel CCD camera with 33 µm camera pixel size. For comparable resolution settings, this new CCD camera has a greater well depth than the previous camera used in the ProXPRESS I instrument, allowing it to capture more light before saturation and consequently delivering a greater dynamic range and true 16-bit images. Increase in signal-to-noise, reduced exposure time and shorter processing time are achievable by binning pixels on the CCD chip. Thus, in addition to acquiring images using the 33 µm pixel size, 66, 100, 133, 166 and 200 µm settings may be employed by binning, allowing selection of the most appropriate balance between speed and spatial resolution for a particular imaging application. (A.) A portion of a variable frequency resolution target (Edmund Scientific) employed to determine the spatial resolution of the imaging system. (B.) Intensity line traces demonstrate that the instrument is capable of resolving 15 lines per mm. *The resolution of the instrument is thus competitive with laser-based gel scanners and superior to fixed CCD camera imaging systems.*



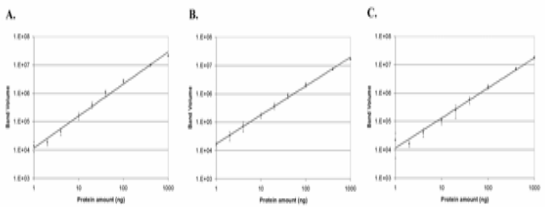
5 Imaging fluorescent and colored stains

A cross-section of representative protein stains is readily detectable using the ProXPRESS 2D imager. A dilution series of broad range molecular weight markers (1 µg-1 ng) (Bio-Rad) was evaluated. (A.) Colloidal Coomassie Blue stain (B.) Silver stain (C.) Nile Red stain (D.) SYPRO Orange stain (E.) SYPRO Ruby stain (F.) Pro-Q Diamond stain. Pro-Q Diamond stain detects phosphoproteins, while the other stains detect total protein.



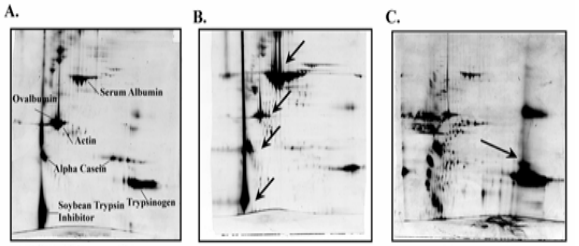
6 Dynamic range analysis

Four replicate gels were run with a dilution series of test proteins loaded in amounts extending over a 4-order of magnitude range and stained with SYPRO Ruby dye. A linear relationship between protein amount and band volume was observed, as shown below. The test proteins used in the analysis were (A.) bovine serum albumin, (B.) chicken egg white lysozyme and (C.) bovine pancreas trypsinogen. The correlation coefficients for the test proteins, was 0.9921, 0.9985 and 0.9856, respectively. The limit of detection was defined to be a signal-to-noise ratio greater than 3. By this criterion, three of the four gels in the study showed a detectable amount of bovine serum albumin at the 0.4 ng level.



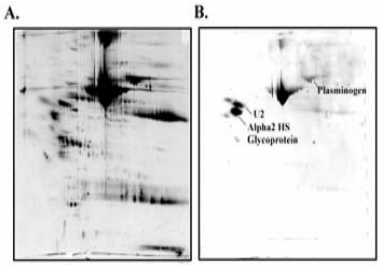
7 Protein pre-fractionation

ProXPRESSION acidic and basic fractionation kits (PerkinElmer), containing ion exchange spin columns, were used to enrich subsets of test proteins. (A.) Unfractionated test protein mixture. (B.) Enriched acidic fraction. (C.) Enriched basic fraction. Enriched proteins are indicated with arrows. Successful application of the fractionation devices and imaging system has revealed uniquely expressed proteins in brain samples from Alzheimer's disease patients (Lopez et al, 2004).



8 Multiplexed detection of phosphoproteins

Human serum proteins separated by 2-D gel electrophoresis, followed by staining for phosphoproteins and total protein. (A). SYPRO Ruby dye stained protein profile. (B.) Pro-Q Diamond dye stained phosphoprotein profile. Several prominent phosphoproteins are indicated in (B). The ProXPRESS 2D Imager is well-suited to multiplexed analysis using fluorescent dyes.



9 Conclusions

The new ProXPRESS 2D imager provides high resolution gel images that are competitive with laser-based scanners in terms of resolution, quantitative accuracy and dynamic range. The instrument is more sensitive and faster than the previously offered ProXPRESS I instrument. The main advantage of the ProXPRESS 2D imaging system is the ability to modify the illumination wavelength to match the excitation maxima of selected fluorophores. Laser-based gel scanners and lower resolution fixed CCD camera systems with gas discharge transilluminators produce restricted wavelengths of light that do not always well match specific dye spectra. Tailoring illumination wavelengths is particularly important for multiplexed imaging applications in proteomics.

References

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